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Flagfish were exposed to cadmium and zinc as individual metals and as mixtures (4.8-8.5 µg Cd/liter and 73.4-139 µg Zn/liter) through one complete life cycle in Lake Superior water (45 mg/liter total hardness). Cadmium and zinc did not act additively at sublethal concentrations when combined as mixtures; however, a joint action of the toxicants was indicated. Effects on survival showed that the toxicity of cadmium and zinc mixtures was little if any greater than the toxicity of zinc alone. Mechanisms of zinc toxicity in this test were similar to those in previous chronic tests of individual metals, indicating that the presence of cadmium did not influence the mode of action of zinc. Comparisons between metal residues in fish exposed to each individual metal or to the metal mixtures showed that the uptake of one metal was not influenced by the presence of the other.

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Chronic Effects of Cadmium and Zinc Mixtures on Flagfish (*Jordanella floridae*)

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ABSTRACT

Flagfish were exposed to cadmium and zinc as individual metals and as mixtures (4.3–8.5 μg Cd/liter and 73.4–139 μg Zn/liter) through one complete life cycle in Lake Superior water (45 mg/liter total hardness). Cadmium and zinc did not act additively at sublethal concentrations when combined as mixtures; however, a joint action of the toxicants was indicated. Effects on survival showed that the toxicity of cadmium and zinc mixtures was little if any greater than the toxicity of zinc alone. Mechanisms of zinc toxicity in this test were similar to those in previous chronic tests of individual metals, indicating that the presence of cadmium did not influence the mode of action of zinc. Comparisons between metal residues in fish exposed to each individual metal or to the metal mixtures showed that the uptake of one metal was not influenced by the presence of the other.

The close chemical properties and similarities of cadmium and zinc result in the common occurrence of these two metals together in the environment (Friberg et al. 1971). Mixtures of these pollutants and of other toxic or potentially toxic substances occur in natural waters from industrial discharge of waste products. A knowledge of mixture toxicity is important in determining water-quality criteria since interactions may increase or decrease toxicity depending upon the nature of the individual pollutants (Sprague 1970).

The evaluation and prediction of mixture toxicity to aquatic life have been studied on a short-term basis (Lloyd 1961; Herbert and Van Dyke 1964; Herbert and Shurben 1964; Sprague and Ramsay 1965; Saunders and Sprague 1967; Brown 1968; Brown and Dalton 1970; Thorp and Lake 1974; and others). Little information, however, is available concerning the chronic toxicity of mixtures other than the work of Eaton (1973) with fathead minnows (*Pimephales promelas*).

The objective of this study was to investigate the toxicity of cadmium and zinc mixtures to the flagfish (*Jordanella floridae*) by studying the effects of these metals on all developmental stages of the life cycle and to determine the combined effects of cadmium and zinc on bioaccumulation. The flagfish was utilized in this test because individual chronic effects of cadmium and zinc on this species have been previously determined in the same water (Spehar 1976).

METHODS

Water Characteristics

Untreated Lake Superior water was used in the test at 25 ± 2 C. Chemical characteristics of the test water were determined weekly according to methods described by the American Public Health Association et al. (1975). Means and ranges (in parentheses) for these measurements were (milligrams per liter): dissolved oxygen 8.2 (7.7–8.7); hardness as $CaCO_3$, 45 (43–46); alkalinity as $CaCO_3$, 43 (40–46); and acidity as $CaCO_3$, 2.2 (1.6–3.2). The pH ranged from 7.1 to 7.9.

Exposure System

The exposure system consisted of a multichannel toxicant injection system (DeFoe 1975), which delivered one concentration of each metal and three concentrations of metal mixtures to duplicate exposure chambers. Spawning chambers were glass aquaria 30 × 60 × 30 cm, with a water volume of 41 liters. Larval chambers were 15 × 30 × 20 cm and contained 7 liters of water. Flow rate to each spawning chamber was 10 liter/h providing 95% replacement of test water every 13 h (Sprague 1969).

Fluorescent bulbs provided a light intensity of 60 and 200 lm of light to adult and larval chambers, respectively. An automatically controlled 16-h photoperiod was used.

Toxicant Solution

Stock solutions were prepared by dissolving reagent grade $CdCl_2$ and $ZnSO_4 \cdot 7H_2O$

TABLE 1.—*Survival and growth of flagfish (*Jordanella floridae*) exposed to mixtures of cadmium and zinc. Asterisk (*) denotes mean values are significantly less than the controls according to Dunnett's procedure (P = 0.05).*

Item	Measured Cd (above) and Zn concentrations (µg/liter)											
	0.1 ± 0.04[a] 139.0 ± 8.4[b]		8.5 ± 0.8 127.0 ± 18.9		6.3 ± 0.6 105.0 ± 14.0		4.3 ± 0.4 73.4 ± 11.5		8.5 ± 0.9 4.5 ± 1.9		(control) 0.1 ± 0.1 4.1 ± 2.2	
	A	B[c]	A	B	A	B	A	B	A	B	A	B
	6[d]		5		4		3		2		1	
14 days												
Survival (%)[e]	0 *	0	0 *	0	20 *	0	20 *	0	80	80	100	90
Survival (%)[f]	83 *	87	100	100	100	100	100	97	80 *	87	97	97
30 days												
Survival (%)[f]	77 *	83	97	97	93	97	100	87	73 *	80	93	97
Mean weight (g)	0.12 ±0.03	0.12 ±0.04	0.11 ±0.02	0.14 ±0.04	0.14 ±0.03	0.12 ±0.02	0.14 ±0.02	0.14 ±0.04	0.12 ±0.03	0.13 ±0.03	0.13 ±0.03	0.11 ±0.03
30 days (F_1)												
Survival (%)	60	95	95	60	100	100	90	100	75	70	90	90
Mean weight (g)	0.25 ±0.11	0.25 ±0.05	0.18 ±0.06	0.26 ±0.07	0.29 ±0.07	0.23 ±0.06	0.26 ±0.07	0.21 ±0.05	0.22 ±0.07	0.35 ±0.11	0.30 ±0.07	0.22 ±0.04

[a] Mean ± SD for cadmium.
[b] Mean ± SD for zinc.
[c] Duplicate chamber.
[d] Chamber number.
[e] Fish not exposed as embryos.
[f] Fish initially exposed as embryos.

in acidified deionized water and were introduced to the diluter by an injection system utilizing 50-ml gas chromatograph syringes (DeFoe 1975). Individual and mixture concentrations of the metals were based on "effect" and "no effect" concentrations derived from previous exposures of flagfish to these metals (Spehar 1976). Weekly composite samples of the test water were analyzed by atomic absorption spectrophotometry. Measurements of cadmium and zinc concentrations are included in Tables 1 and 2. Individual metals are represented by chambers 2 and 6 and metal mixtures by chambers 3, 4, and 5. To verify the accuracy of the method of analysis, known amounts of each metal were added to control water to obtain percentage recoveries. Mean percentage recoveries and standard deviations for 16 spiked cadmium and zinc samples were 90 ± 1.0 and 100 ± 2.0, respectively. Additionally, one set of unacidified samples

TABLE 2.—*Growth and reproduction of flagfish exposed to mixtures of cadmium and zinc for 100 days.*

Item	Measured Cd (above) and Zn concentration (µg/liter)											
	0.1 ± 0.04[a] 139.0 ± 8.4[b]		8.5 ± 0.8 127.0 ± 18.9		6.3 ± 0.6 105.0 ± 14.0		4.3 ± 0.4 73.4 ± 11.5		8.5 ± 0.9 4.5 ± 1.9		(control) 0.1 ± 0.1 4.1 ± 2.2	
	A	B[c]	A	B	A	B	A	B	A	B	A	B
	6[d]		5		4		3		2		1	
Males/females at termination	0/3	2/4	2/3	2/4	1/2	2/4	2/4	2/5	2/4	2/5	2/5	2/3
Mean weight (g)												
Male		3.0	3.6	2.3	3.8	2.4	2.2	2.9	2.4	2.2	2.8	2.8
Female	1.1	1.2	1.3	1.3	1.3	1.1	1.5	1.3	1.3	1.2	1.4	1.3
Mean spawnings per female	5.7	5.0	4.3	2.8	0	4.5	3.3	3.2	7.3	4.4	6.4	8.0
Total embryos produced	880	1,119	515	672	0	737	519	490	2,439	1,479	1,249	1,037
Mean embryos per females	293	280	172	168	0	184	130	98	610	296	250	346
Mean hatchability (%)	78 (10)[e]		85 (9)		81 (6)		60 (10)		71 (11)		84 (16)	

[a] Mean ± SD for cadmium.
[b] Mean ± SD for zinc.
[c] Duplicate chamber.
[d] Chamber number.
[e] Parentheses enclose number of spawnings checked for hatchability.

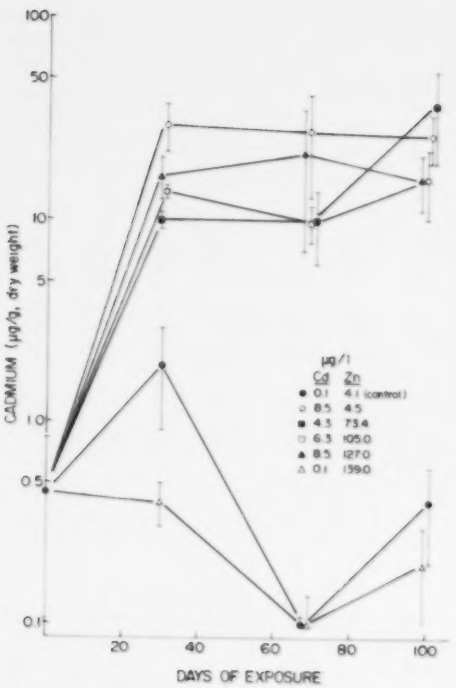

FIGURE 1.—*Cadmium residues from whole-bodies of flagfish exposed to mixtures of cadmium and zinc. Each symbol and bar indicates the mean and standard deviation of five samples.*

for each metal was filtered through a 0.1-µm filter to characterize the portion of dissolved metal. The amount of dissolved cadmium and zinc ranged from 90% to 100% of the total.

Biological Procedures

To initiate the testing, enough embryos were incubated in each exposure concentration to provide thirty 1-day-old larvae to each duplicate chamber. In addition, ten 1-day-old larvae not exposed as embryos (incubated in the controls) were placed in each duplicate chamber (separated by a stainless steel screen partition) and exposed for 2 weeks so comparisons could be made to determine the effect of embryo acclimation on larval sensitivity.

Larvae were fed brine shrimp nauplii four times a day for the first 30 days. After this time all fish were randomly selected and reduced to 15 per chamber and fed a diet of frozen brine shrimp twice a day. After approximately 40 days, two spawning substrates (Spehar 1976) were placed in each tank to initiate spawning. The number of fish per chamber was further reduced to two males and five females after males began displaying territorial behavior, about the 60th day of the test.

Reproduction studies were started after 30 or more embryos appeared on any one substrate and were continued for a period of 45 days. At this time 30–50 embryos were removed from the substrates, placed in oscillating egg cups, and incubated in the test water. All embryos not incubated for hatchability determinations were counted and discarded.

Twenty newly hatched (F_1) larvae from each toxicant concentration were randomly selected and transferred to corresponding duplicate larval chambers for 30-day growth and survival exposure.

Residue Analysis

Whole fish from each concentration were analyzed for cadmium and zinc (dry weight) after 30, 70, and 100 days (end of test) of exposure. Five fish were analyzed per concentration for both cadmium and zinc. Embryos hatched in control water were also analyzed for both metals to determine a baseline residue value. A 98–101% recovery of the metals for 19 cadmium and 13 zinc samples was obtained by using a wet digestion, atomic absorption spectrophotometric analysis (Leonard 1971).

Statistical Analysis

For statistical evaluation, survival, growth, reproduction, and residue accumulation data were subjected to an analysis of variance ($P = 0.05$) and Dunnett's two-tailed comparison of treatment means to control means ($P = 0.05$) (Steel and Torrie 1960).

RESULTS

Toxicity

Survival of larvae not exposed as embryos was significantly reduced from that of the controls in all concentrations except the individual cadmium concentration (8.5 µg/liter, chamber 2) after 14 days of exposure

(Table 1). Survival of larvae initially exposed as embryos was significantly reduced in both individual metal concentrations (8.5 μg Cd/liter and 139 μg Zn/liter; chambers 2 and 6) but not in the mixture concentrations (chambers 3, 4, and 5), after 14 and 30 days of exposure. Growth of larvae exposed for 30 days was not significantly different from that of controls.

Although sexual maturity of the fish apparently was not delayed in any of the exposure concentrations, normal spawning behavior (courting, aggressive territoriality, and female acceptance of males) was noticeably decreased in all exposure concentrations from that of control fish. Exposed fish were usually found near the bottoms of tanks and became extremely hyperactive when being observed, during feeding, or just before spawning. Hyperactivity consisted of uncontrolled swimming movements, convulsions, and loss of equilibrium and was more noticeable in males than females.

After 100 days of exposure, growth and reproduction were not significantly different between exposed flagfish and the controls (Table 2). However, mean spawnings per female and embryo production was lower in all mixture concentrations (chambers 3, 4, and 5) than in the controls or in the individual metal concentrations. No spawning took place in one duplicate chamber of the intermediate mixture concentration (chamber 4). Significant decreases in embryo hatchability and survival and growth of F_1 larvae (Table 1) exposed for 30 days were not observed in any of the concentrations.

Accumulation

Fish exposed for 30 days to both individual and mixture concentrations of cadmium and zinc contained greater amounts of these metals than the controls (Figs. 1 and 2). However, both cadmium and zinc residues appeared to reach an equilibrium on a microgram-per-gram basis by the 30th day of exposure. Metal residue concentrations in fish exposed to each individual metal and to the metal mixtures were similar after 30, 70, and 100 days of exposure.

Cadmium and zinc residues in progeny (F_1) exposed for 30 days to individual and mixture concentrations were similar to those in parental fish exposed for the same period.

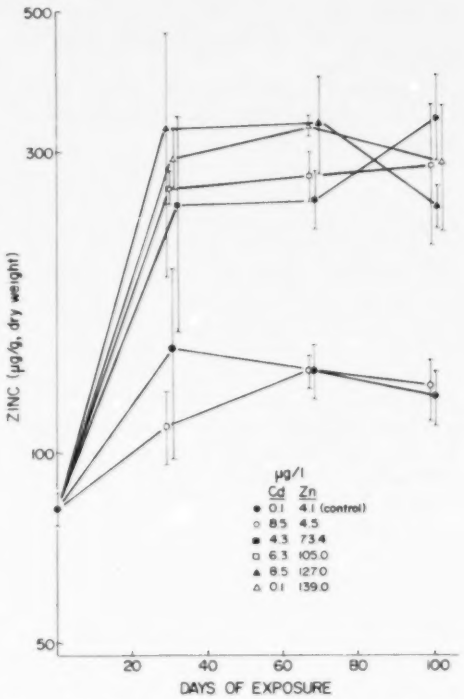

FIGURE 2.—*Zinc residues from whole-bodies of flagfish exposed to mixtures of cadmium and zinc. Each symbol and bar indicates the mean and standard deviation of five samples.*

DISCUSSION

Survival of flagfish larvae not previously exposed as embryos was significantly reduced in all concentrations except that containing cadmium alone. Concentrations causing this effect were similar to zinc concentrations causing decreases in larval survival in a previous exposure with zinc only (Spehar 1976). Since larvae not exposed as embryos were a sensitive indicator of toxicity in both studies and were affected to a similar degree by individual and mixture concentrations containing zinc, it can be concluded that the chronic toxicity of cadmium and zinc mixtures was little if any greater than the toxicity of zinc alone. Eaton (1973), using embryo production as the basis

of effect, observed that zinc was the toxic component of a trimetal mixture with fathead minnows.

In earlier chronic tests with zinc involving flagfish (Spehar 1976) and fathead minnows (Brungs 1969), survival and reproduction, respectively, were the most sensitive indicators of zinc toxicity. In the present test and in the mixture test conducted by Eaton (1973) with fathead minnows, survival and reproduction, respectively, were again the most sensitive indicators of toxicity indicating that the presence of cadmium did not influence the mode of action of zinc.

Larvae initially exposed as embryos to the individual zinc concentration and to mixtures of cadmium and zinc were much more tolerant than those not previously exposed, indicating acclimation during embryo exposure. The result observed in the mixture concentrations was probably due to zinc since similar results were observed at these concentrations in earlier chronic zinc tests but not in a test with cadmium. The acclimation of larvae was attributed to the fact that embryos were exposed to only small amounts of zinc (3% of the total accumulated zinc compared to 70% in the chorion: Wedemeyer 1968) which they could metabolize and become acclimated to. Manner et al. (1977) believed that changes in the ultrastructure and thickness of the chorion during embryogenesis could account for changes in the susceptability of the embryo to toxic agents.

Survival of larvae exposed as embryos was significantly decreased in both individual cadmium and zinc concentrations, but was not decreased in mixtures of these concentrations suggesting that these metals acted antagonistically on this phase of the life cycle. However, the interpretation of this result is complex because significant effects on survival were not observed at the same individual metal concentrations in tests with progeny (F_1) or in earlier chronic tests (Spehar 1976). Survival percentages in both tests were approximately the same. Differences in the levels of susceptibility of test fish and/or the variability of response between duplicate chambers might help account for the significant survival decreases in one test and not in the other.

In this test, mixtures of cadmium and zinc may have caused greater decreases in reproduction (number of spawnings per female and embryo production) than corresponding individual metal concentrations. However, additive effects could not be shown because of the lack of statistically significant differences ($P = 0.05$) between the exposure concentrations and the control. Eaton (1973) concluded that the toxicity of a mixture of copper, cadmium, and zinc could not be predicted closely from the results of individual toxicant chronic tests by assuming that their toxic effects were totally additive.

Residues of cadmium and zinc accumulated when fish were exposed to both individual and mixture concentrations and appeared to reach an equilibrium on a microgram-per-gram basis. The same effect was noted for cadmium residues in flagfish (Spehar 1976) and brook trout (Benoit et al. 1976). Comparison between metal residues in fish exposed to cadmium and zinc, or to mixtures of these, showed that the uptake of one metal was not influenced by the presence of the other (Figs. 1 and 2). This result might have been expected since more than additive effects were not observed on toxicity. Hutchinson and Czyrska (1972) found that when cadmium and zinc acted jointly in causing inhibitory growth effects on aquatic plants, the uptake of one metal was increased by the presence of the other.

Previous acute studies with cadmium and zinc by Bandt (1946), Eaton (1973), and Thorp and Lake (1974) showed that these metals acted additively in their effects on various aquatic organisms. The results of this study and those observed by Eaton (1973) with fathead minnows indicate that cadmium and zinc and copper, cadmium, and zinc, respectively, did not act additively in chronic tests at sublethal levels when present as mixtures. However, both studies indicate that these metals interacted to some degree. Hutchinson and Czyrska (1972) reported that mixtures of cadmium and zinc caused greater growth inhibition in plants than did either individual metal at sublethal concentrations. Sprague et al. (1965) found that metals such as copper and zinc in a mixture of concentrations equal to

1% of their lethal thresholds were more than additive in causing an avoidance response in Atlantic salmon parr. Results of recent studies by Biesinger, Christensen, and Fiandt (in preparation, Environmental Research Laboratory, Duluth, Minnesota) have shown that mixtures of cadmium and zinc, cadmium and mercury, and zinc and mercury caused significant decreases in reproductive success of *Daphnia magna* at concentrations that had no effect as individual metals in chronic tests. Additional studies by these authors showed that mixtures of up to 21 metals caused similar results.

Although the interaction of cadmium and zinc in this test would not have affected the recommended "safe" levels for these metals in water (U.S. Environmental Protection Agency 1976) results of the above mentioned experiments illustrate the danger of setting water quality standards on the basis of individually tested toxicants. The interaction of toxicants is an important factor to consider when setting individual pollutant standards since it is unlikely that toxicants ever occur individually in the environment. Additional chronic tests or tests involving the use of sensitive life stages of organisms (McKim 1977) are urgently needed to gain more knowledge of interactions and their toxic effects if we are to predict "safe" concentrations for aquatic life.

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